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Soil respiration and plant growth across a chronosequence of tallgrass prairie reconstructions

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**Soil respiration and plant growth across a chronosequence
of tallgrass prairie reconstructions**

by

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CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

Land use change through human activity has dramatically altered the global carbon cycle (Houghton et al. 1983). In the Midwestern U.S.A., agricultural expansion has driven the widespread conversion of native tallgrass prairie ecosystems into a landscape presently dominated by annual cropping systems, primarily corn and soybean. While these cropping systems have been designed to maximize the productive potential of aboveground plant biomass for the harvest of food and fuel crops, they have reduced the capacity of agricultural land to sustain historic levels of soil carbon (e.g. Tiessen et al. 1982; Huggins et al. 1998). Soil carbon loss with the cultivation of tallgrass prairie soils has been estimated to range from 40-60% of native levels (Huggins et al. 1998). This process of soil carbon depletion has been driven by a combination of agricultural management strategies that have decreased belowground carbon inputs, accelerated rates of decomposition, and increased soil erosion (Buyanovsky et al. 1987; Huggins et al. 2007).

Largely as a result of anthropogenic induced increases in atmospheric CO_2 concentrations, attention has been given to quantifying carbon stocks and flows across both grassland and agricultural ecosystems (Brye et al. 2002; Paustian et al. 2000). In historical grassland regions, efforts have been directed toward quantifying the potential of reconstructed grassland ecosystems within agricultural landscapes to increase the carbon storage capacity of soils (Paustian et al. 1998; Rees et al. 2005; Kucharik et al. 2001). In the past, federal programs, such as the Conservation Reserve Program, have facilitated the

planting of perennial grasslands and have served to retire highly erodible lands and enhance soil carbon stocks (Gebhart et al. 1994; Burke et al. 1995; Baer et al. 2002). Tallgrass prairie reconstructions have introduced a level of native plant diversity that has led to the study of community and system level processes that influence soil carbon accumulation in formerly cultivated land (Camill et al. 2004; Jastrow 1987). Estimates of the rate of change in soil organic carbon with grassland reconstruction are highly variable, depending on pre-existing soil carbon conditions and the difficultly in distinguishing small increases from relatively large backround pools (McLaughan 2006).

The accumulation of soil organic matter in reconstructed tallgrass prairie ecosystems is largely dependent on the storage of root-derived carbon. Tallgrass prairies allocate a large percentage of fixed carbon belowground relative to annual crops (Jackson et al. 1996) and the distribution and cycling of plant biomass belowground is in part responsible for the distinction of carbon rich, tallgrass prairie derived soils (Rice et al. 1998). However, our present understanding of belowground carbon cycling in tallgrass prairie reconstructions is limited by the difficulty in quantifying plant root dynamics. Estimates of belowground plant growth within grassland reconstructions and differences in plant carbon allocation among annual crops and young grasslands will provide critical knowledge towards predicting how reconstructions alter the magnitude and rate of soil carbon cycling processes.

Meanwhile, the consequence of tallgrass prairie age on changes in the quantity and quality of belowground plant inputs remains a major knowledge gap in the study of tallgrass prairie ecosystem development. Tallgrass prairie plantings have been shown to accelerate plant community succession relative to natural rates of succession (Camill et al. 2004), leading to relatively fast changes in both the quantity and quality of belowground inputs.

While poorly documented, patterns in belowground plant production with age will have important implications for the rate of carbon accumulation in reconstructed grasslands.

Our understanding of the rate of change in soil carbon with grassland development must extend beyond changes in belowground production to consider the processes that influence the fate of carbon allocated belowground. Studies in reconstructed grasslands have frequently considered the quality of root-derived inputs and their relative availability for soil microbial decomposition (Burke et al. 1995; Baer et al. 2002), while little work has actually quantified in-situ rates of the quantity of carbon respired from the soil to the atmosphere. The process of soil respiration represents a combination of CO_2 respired through root and rhizosphere metabolism and the microbial decomposition of soil organic matter. Soil respiration and its sensitivity to environmental change (Kirschbaum 2000) could ultimately determine the capacity of reconstructed ecosystems to accumulate soil carbon with age.

Predictions of soil carbon cycling in tallgrass prairie reconstructions should also take into account that these grasslands will be planted within a changing global environment, where a rise in atmospheric CO_2 concentrations will alter both climatic conditions and plant growth and allocation (Hungate et al. 1997). Globally, the annual flux of carbon produced by soils has been estimated to be 10 times that of fossil fuel combustion (Raich and Potter 1995). In this context, efforts to quantify the magnitude of change in soil respiration with grassland reconstruction should be incorporated with an assessment of the climatic and biological drivers of this process. Predictions of soil respiration in grasslands could require accounting for differences in carbon supply to roots and the quantity and quality of detritus, where variability in the response of soil respiration to climatic change, i.e. rising temperatures, could also depend on substrate conditions (Hibbard et al. 2005). Identifying the

controls on soil respiration in reconstructed grasslands over time is necessary if we are to predict future trends in the soil carbon budget in these ecosystems.

Root production and soil respiration are critical ecosystem processes within the tallgrass prairie carbon budget; however we have limited knowledge of the magnitude and directional change of either process with the development of tallgrass prairies planted into formerly cultivated ecosystems. This research was designed to quantify changes in carbon fluxes with the development of tallgrass prairie plantings in cultivated land and the controls on soil carbon cycling across a reconstructed landscape. This study used tallgrass prairie reconstructions along a 12 yr chronosequence in central Iowa, USA, including a no-till soybean field, to assess how ecosystem carbon fluxes changed with prairie age. The first objective of this research was to quantify changes in soil respiration, root biomass, above and belowground plant production, and the quality of root derived inputs (i.e. C:N) with tallgrass prairie age. Secondly, this study was designed to identify the importance and interaction of climatic and biological controls in predictions of soil respiration across a landscape of annual cropping systems and grassland reconstructions.

THESIS ORGANIZATION

This thesis is composed of three chapters. Chapter I is a concise introduction to the research problem. Chapter II is the main body of the thesis and was written with intent for publication in the journal *Ecosystems*. Chapter III summarizes the conclusions of the research and outlines suggestions for future research directions.

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CHAPTER 2: SOIL RESPIRATION AND PLANT GROWTH ACROSS A CHRONOSEQUENCE OF TALLGRASS PRAIRIE RECONSTRUCTIONS

A paper to be submitted to the journal *Ecosystems*

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ABSTRACT

An understanding of changes in soil respiration (R_s) and plant growth in tallgrass prairies planted into formerly cultivated land is critical if we are to predict the effects of grassland reconstructions on belowground carbon cycling. In addition, predicting changes in the ecosystem carbon balance in grassland reconstructions will require identifying the climatic and biological controls on R_s across a landscape of cultivated and reconstructed grassland ecosystems. This study used a 12 yr chronosequence of tallgrass prairie reconstructions in central Iowa, including a no-till soybean field (age 0), to quantify the relationship between tallgrass prairie age, R_s, root biomass, root ingrowth, and aboveground production. We also assessed the strength and interaction of soil temperature and soil moisture in predictions of R_s across the chronosequence. Linear regressions showed a significant increase in standing root biomass carbon ($R^2 = 0.89$) and growing season R_s ($R^2 = 0.83$) with prairie reconstruction age while changes in aboveground production and root ingrowth were less predictable. Growing season (gs) R_s represented the largest carbon flux

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[1] Primary researcher and author

among prairie ages, ranging from 624 g C m^{-2} gs^{-1} in the soybean cropping system to 939 g C m^{-2} gs^{-1} in the oldest reconstruction (age 12), and was positively correlated with changes in root biomass. Among all tallgrass prairie reconstructions there was a strong, positive relationship between soil temperature and R$_s$ (R^2 = 0.80 to R^2 = 0.91) while the effect of soil moisture was greatest for the youngest prairie (age 4). Soil temperature was less correlated with R$_s$ in the no-till soybean field (R^2 = 0.40) and the inclusion of soil moisture added limited additional predictive power (R^2 = 0.48). Our findings indicate that an increase in cumulative R$_s$ with prairie reconstruction age was related to the interaction of soil temperature and the accumulation of root biomass with young grassland development.

INTRODUCTION

Land use change through agricultural conversion has dramatically altered the carbon cycle within terrestrial ecosystems (Paustian et al. 1998). The loss of tallgrass prairie ecosystems in the Great Plains region of the U.S. to annual cropping systems led to a historic decline in soil C and N stocks (Huggins et al. 1998) and has altered the processes that contribute to soil organic carbon accumulation (Buyanovsky et al. 1987). Our attempts to restore ecosystem functions, including soil organic matter accumulation, to agriculturally dominated landscapes through grassland reconstructions demands a greater understanding of belowground carbon cycling within these intensively managed landscapes.

The accumulation of soil carbon in reconstructed grasslands planted into formerly cultivated land will largely depend on the quantity and quality of carbon allocated belowground (Kucharik et. al 2001; Baer et al. 2002; Camill et al. 2004). However, the

response of soil respiration or "soil CO_2 efflux" (R_s), a measure of total soil metabolism that includes both root and rhizosphere and microbial respiration, will also have an important consequence on the ecosystem carbon balance (Valentini et al. 2000). Within tallgrass prairie ecosystems, R_s has shown to account for a majority of total ecosystem respiration (Ham et al. 1995) and Brye et al. (2002) suggested that the carbon balance could be more sensitive to fluctuations in soil respiration than net primary production. Despite their importance within the grassland carbon cycle, patterns and controls on plant root allocation and R_s within grassland reconstructions are poorly understood and there have been few attempts to assess how these processes change with grassland age. Therefore, understanding how R_s changes over time in tallgrass prairie reconstructions is critical to predictions of their capacity to enhance ecosystem carbon storage.

Grasslands planted into cultivated ecosystems over time have offered a chronosequence approach for monitoring changes in soil physical characteristics (Jastrow 1987), carbon and nitrogen dynamics (Burke et al. 1995; Baer et al. 2002; Camill et al. 2004; Kucharik et al. 2001), microbial and fungal community composition (Allison et al. 2005), and plant community composition (Jastrow 1987; Camill et. al 2004). While small changes in large pools of soil carbon can be difficult to detect in the short term (Brye et al. 2002; Camill et al. 2004), studies of tallgrass prairie reconstructions suggest significant changes in plant carbon allocation with young grassland development. For example, C_4 dominated grassland plantings have shown a linear increase in standing root biomass and the C:N ratio of root tissue with age (Baer et al. 2002). Similarly, Camill et al. (2004) found an increase in belowground production in tallgrass prairie after 3 growing seasons in accordance with a shift in the plant community towards C_4 grass dominance. Trends in aboveground

productivity have been less consistent (Camill et al. 2004; Jastrow 1987) given the

predominance of management, such as fire and mowing, in directly regulating aboveground

stocks (Baer et al. 2002). Overall, these previous studies suggest significant changes in the

quantity and quality of belowground biomass and production across young grassland

chronosequences, however estimates of belowground production remain limited and none of

these studies quantified changes in soil respiration over time. To date, R_s in tallgrass prairies

has only been measured in mature tallgrass prairie ecosystems (e.g. Kucera and Kirkham

1971; Mielnick and Dugas 2000; Brye et al. 2002; Knapp et al. 1998; Wagai et al.1998;

Franzluebbers et al. 2002). Accounting for changes in R_s with seasonal and annual measures

of plant allocation across different age grassland reconstructions will improve our

understanding of the processes that influence the directional change in soil carbon storage

over time.

Despite the significance of R_s as the primary component of ecosystem metabolism

and as the second largest flux in the terrestrial carbon cycle (Raich and Schlesinger 1992), we

have a limited understanding of the controls on R_s at local and regional scales. Identifying the

controls on R_s is important if we are to predict the response of soil respiration to future land

use and climate change. While soil temperature and moisture have been used to predict R_s

within tallgrass prairie ecosystems (Kucera and Kirkham 1971; Mielnick and Dugas 2000),

these abiotic controls have also shown limitations when predicting across local grassland

ecosystems (Bremer and Ham 2002; Craine and Wedin 2002) and recent attention has been

given to differences in substrate supply (Hibbard et al. 2005). Variability in the relationship

between abiotic controls and R_s across sites has been associated with differences in carbon

supply to roots or the quantity and quality of detritus available for microbial decomposition

(Ryan and Law 2005). In both native and experimental grasslands, measures of canopy

photosynthesis, such as leaf area index and ANPP, have been used as a surrogate for

belowground carbohydrate supply to improve models of R_s (Bremer and Ham 2002; Craine

et al. 1999; Franzluebbers et al. 2002), while others have shown that direct measurements of

root biomass and root biomass N are more appropriate (Dornbush and Raich 2006).

Dornbush and Raich (2006) suggested that a decoupling of above and belowground plant

biomass allocation in grasslands could provide support for the importance of incorporating

measures of root activity into models developed for predicting R_s.

Reconstructed tallgrass prairies could provide a gradient in root biomass,

belowground and aboveground production, and root tissue nitrogen content that would

provide insight into the importance of these different measures of substrate quantity and

quality and their interaction with soil microclimate conditions in predictions of R_s across

grasslands. For example, changes in root nitrogen concentrations with plant community

succession (Baer et al. 2002) could control total root nitrogen content and override the

positive relationship between root nitrogen and root respiration (Bahn et al. 2006), potentially

limiting the effect of increasing plant root biomass on R_s. Differences in soil microclimate

driven by changes in vegetation structure (Raich and Tufekcioglu 2000) could also influence

the relationship between plant production and R_s across tallgrass prairie plant communities.

Additionally, predictions of R_s with land use change, such as prairie reconstructions

in former agricultural lands, could be improved when considering differences in plant

allocation between perennial grasslands and annual croplands. Annual cropping systems have

been designed to maximize aboveground production while grasslands allocate greater than

50% of productivity belowground (Brye et al. 2002; Buyanovsky et al. 1987). Differences in

R_s among a reconstructed tallgrass prairie and a corn cropping system have been poorly predicted by soil temperature and moisture and Wagai et al. (1998) suggested that higher rates in prairie were related to greater fine root and microbial biomass. Models of R_s within cultivated ecosystems and in comparison to grasslands could be improved by taking into account differences in belowground plant activity (Raich and Mora 2005) and/or changes in perennial root biomass with ecosystem development.

Large-scale tallgrass prairie reconstructions within landscapes dominated by annual cropping systems present a unique experimental framework for studying the impact of native perennial plantings on both R_s and plant production. This study used a chronosequence of tallgrass prairie reconstructions to investigate how time since tallgrass prairie planting affects seasonal and annual plant growth and R_s and to assess soil temperature, moisture, and plant growth as determinants of R_s. Specifically, this study was designed to test the following hypotheses:

> (1) Cumulative R_s, standing root biomass, root ingrowth, aboveground production, and the ratio of root ingrowth to aboveground plant production will increase with tallgrass prairie age over the 12 yr chronosequence;
>
> (2) Seasonal variation in R_s is strongly related to seasonal changes in soil temperature and moisture within grasslands;
>
> (3) Changes in the relationship between R_s and soil microclimate with reconstruction age are related to differences in root biomass and root ingrowth (e.g. root C and N).

Information on R_s and belowground biomass and production combined with measurements of changes in soil carbon pools will enhance understanding of the carbon transformations

between the plant, soil, and atmosphere with tallgrass prairie establishment and provide insight into the mechanisms that influence soil carbon accumulation over time.

METHODS

Study Site

This study was conducted in 2006 at the Neal Smith National Wildlife Refuge (NSNWR) in Jasper County, central Iowa (41˚33′N, 93˚17′W). The refuge was established in 1991 under the mission of converting over 3,400 ha of an agriculturally dominated landscape to pre-European settlement tallgrass prairie and oak-savanna ecosystems. Presently, the refuge consists of a mosaic of reconstructions and agricultural land uses with approximately 1,200 ha planted to tallgrass prairie through annually successive plantings. This region has a mean annual precipitation (based on 1971-2000 average) of 87.4 cm and mean annual temperature of 9.6˚C (MRCC 2007). In 2006, annual precipitation was approximately 64.2 cm and average monthly temperature was 10.8˚C, ranging from -3.0˚C in February to 24.8˚C in July (NESDIS 2007).

This study used five tallgrass prairie plantings and a no-till soybean field (n = 6), ranging in size from 2 to 7.5 ha, to represent a gradient in time of reconstructions that included prairies planted into formerly cropped lands in 2002 (age 4), 2000 (age 6), 1998 (age 8), 1997 (age 9), and 1994 (age 12). Each site was sampled during the 2006 growing season and age was based on the number of growing seasons since planting. Prairie plantings were seeded in the late fall after a soybean crop, therefore we used the predominant land use in the region, a corn-soybean cropping system, to represent a 2006 planting (age = 0). Seed

sources and composition over the chronosequence has changed with refuge expansion and adaptive management. Generally, seed sources shifted from local prairie remnants and outside suppliers (pre-1996) to bulk harvests from within refuge plantings (post-1996). All plantings were supplemented with forb species collected from local remnants. Difficulty establishing cool season grasses led to a modification in seed composition after 1997 with an increase in the use of *Elymus canadensis* as a nurse crop. The soybean field was managed under a standard no-till corn-soybean rotation and soybeans were planted on DOY 127 and harvested on DOY 272.

The plantings used within this study were selected to complement a long-term assessment of changes in soil carbon with tallgrass prairie establishment at NSNWR (Cambardella et al. 2004). The oldest reconstructions were selected in the spring of 2000 based on GIS overlays of refuge plantings and soil mapping units with additional younger sites added in 2005. All plantings were located on either Tama (Fine-silty, mixed, superactive, mesic Typic Argiudoll) or Otley (Fine, smectitic, mesic Oxyaquic Argiudoll) soil series. Both of these map units are deep, well-drained soils developed in loess under grassland vegetation and occur on convex ridge tops and side slopes (ranging from 0 to 9%). Similarity in specific soil characteristics collected in previous work (Cambardella, unpublished) suggests that site comparisons are reasonable (Table 1).

A subsample of the original, replicated chronosequence was selected for this study with additional consideration for refuge management history. While we could not control for the historical burn regime, sites were selected so that time since last burn remained constant. All sites received a prescribed burn in the fall of 2004. None of the sites had been grazed since prairie planting. Within each of the six sites, 10 random plot locations were selected

through ArcView GIS and we used Geographic Position System (GPS) to locate sampling plots in the field.

This prairie chronosequence is subject to the limitations of space-for-time substitution (Pickett 1989). However, consideration of soil characteristics and management history in site selection and sampling design, as stated above, can minimize confounding factors that influence trends due to time. Site selection was based on clearly defined criteria that was chosen *a priori* to minimize intersite variability in variables other than 'time since establishment'. The chronosequence approach has shown to be a reliable measure of successional change (Foster and Tilman 2000) and chronosequence studies have shown to be valuable in developing our understanding of changes in reconstructed grassland structure and function over time (Jastrow 1987; Camill et al. 2004; Baer et al. 2002).

Root Biomass

Standing root biomass (≤ 2 mm) was sampled in the spring of 2006 on DOY 100. One root core (diameter 7.0 cm) was taken at each plot to a depth of 30 cm from a random location in close proximity to each R_s collar within each site (n = 60). Cores in the soybean crop were stratified to account for row and inter-row locations. Root cores were subdivided into three depth intervals prior to processing: 0-10, 10-20, and 20-30 cm. Samples were washed using a hydropneumatic elutriation system (Russell et al. 2004), dried at 65˚C for 24 hours, and sorted from organic debris. No attempt was made to distinguish live from dead roots. Total carbon and nitrogen in root biomass was conducted using a LECO TruSpec CHN Analyzer.

Root Ingrowth

Root production was estimated using the root ingrowth technique (Dornbush 2005; Johnson and Matchett 2001; Russell et al. 2004). For site installation, litter was removed from the soil surface and one soil core (diameter 7 cm) was harvested in close proximity to each soil R_s collar. The soil core was then replaced with a soil-filled root ingrowth tube and covered with surface litter. Root ingrowth tubes were constructed of polypropylene mesh tubes (16-mm^2 holes)(InterNet Inc., Anoka, MN), 7 cm in diameter and 30 cm in length, and sown with mesh bottoms. Each ingrowth tube was filled with soil that was collected on site and sieved (6 mm) to remove roots. Ingrowth cores in the soybean crop were stratified to account for row and inter-row locations. Harvested root ingrowth biomass samples were washed, dried, sorted, and analyzed for total C and N similar to root biomass samples.

Root ingrowth was measured for approximately three months over three overlapping time intervals: DOY 100 through DOY 193 (spring), DOY 157 through DOY 251 (summer), and DOY 221 through DOY 310 (fall). Overlapping ingrowth periods were used based on the methods of Dornbush (2005) to address the disturbance-induced biases associated with root ingrowth methods (Vogt et al. 1998). Root ingrowth during overlapping growth periods, approximately 30 days, was determined based on the average daily production of overlapping ingrowth samplings. Annual root ingrowth (g C or g N m^{-2} yr^{-1}) was estimated by multiplying the average daily growth by the number of days within each period and then summing all ingrowth periods.

Aboveground Production

Aboveground biomass was harvested four times between May and October on an average of 2 month intervals, DOY 134, 178, 226, and 293. A total of eight 0.25 m^2 quadrats

were randomly sampled from each site adjacent to R_s sample plots. All vegetation within the quadrat was clipped, removed, and separated into live and dead fractions. Biomass was dried at 65°C for at least 48 hours, and weighed. Aboveground net primary production (ANPP) (g $m^{-2} yr^{-1}$) in the prairie was based on the sum of positive increments in live and dead biomass (Scurlock et al. 2002; Dornbush and Raich 2006), using the following procedure:

If Δ Live > 0 and Δ Litter > 0, then ANPP = Δ Live + Δ Litter

If Δ Live > 0 and Δ Litter < 0, then ANPP = Δ Live

If Δ Live < 0 and Δ Litter > 0, then ANPP = Δ Live + Δ Litter

If Δ Live < 0 and Δ Litter < 0, then ANPP = 0

If ANPP < 0, then ANPP = 0.

This method attempts to account for growth and death with seasonably variable production in multispecies grasslands. Live biomass harvested on DOY 134 was assumed to be current year's growth and was added to total annual production. Aboveground live biomass harvests in the soybean crop were also conducted on DOY 134, 178, 226, and 293 for direct seasonal comparisons with prairie. Additionally, we collected a separate biomass sample at DOY 244 to account for peak live biomass in the soybean crop. Based on minimal litter accumulation in soybean, ANPP (g $m^{-2} yr^{-1}$) was equivalent to peak live standing crop. Aboveground biomass in all ages was converted to grams of carbon based on an estimate of 43% carbon in plant biomass (Kucharik et al. 2006).

Soil Respiration

Soil surface CO_2 efflux was measured with a custom gas-analysis system, controlled by a Campbell 10X datalogger with an infrared gas analyzer (IRGA, LI-COR 820). This

system is designed for large PVC chambers (506.7 cm^2 soil surface area). Prior to each measurement, CO_2 is scrubbed below ambient concentration to minimize bias associated with CO_2 concentration gradients between the chamber and the air. After scrubbing, the change in concentration was measured every 5 seconds for a 75 second sampling interval through ambient concentration. The IRGA was calibrated at a high (500 ppm CO_2) and low (0 ppm CO_2) standard prior to each measurement period.

Plastic (PVC) collars, 8 cm tall x 25 cm diameter, were permanently installed one week prior to the first sampling period to allow time for collars to equilibrate with the soil and minimize the effects of disturbance (Davidson et al. 2002). Collars (n = 10 per site) were inserted to a depth of 2 cm and included surface litter and plant crowns, accounting for potential high flux that derives from plant crowns (Craine et al. 1999). Standing live vegetation within each collar was clipped at the soil surface 24 hours before sampling when growing conditions required.

R_s measurements were conducted from day-of-year (DOY) 109 through DOY 321, approximately biweekly (n = 15 in each prairie site and n = 13 in the soybean site). Fewer measurements were taken in the soybean field because planting and harvest required removal and replacement of collars. The large number of collars and within-site distance between collars required two consecutive days for each sampling period. The order in which sites were sampled was randomized for each measurement period and measurements took place between the hours of 1000 and 1500. R_s rates (μmol CO_2 m^{-2} sec^{-1}) during this time period were used as representative of the daily average R_s. Based on diurnal measurements conducted on DOY 173 and DOY 306, efflux rates between 1000 and 1500 were within 8% of the daily average (Appendix). A similar agreement between mid-day measurements and

the daily average has been reported by others (Knapp et al. 1998; Kaye et al. 2005), further suggesting that instantaneous measurements at this time period provided a reasonable estimate of the daily average R_s.

Soil temperature at 10 cm was measured simultaneously with R_s measurements with a digital long-stem thermometer attached to the IRGA system. Volumetric soil moisture content (% vol) was measured with a ThetaProbe (Delta-T Devices Inc.) to a depth of 6 cm at four random locations adjacent to each collar immediately after soil CO_2 flux measurements. Millivolt output was converted to volumetric soil moisture content based on calibration standards for mineral soil.

Scaling R_s to Cumulative Growing Season Estimates

Daily average R_s measurements were used to estimate cumulative growing season R_s based on linear interpolation between measurement periods. This estimate assumes that a two week measurement interval is sufficient to capture seasonal variation in R_s. We tested this assumption for DOY 165 through DOY 294 using continuous soil temperature data collected at a nearby upland prairie site within the refuge (T. Sauer, unpublished). Daily soil temperature averages from continuous measurements were correlated with instantaneous temperature measurements when days overlapped (n = 9) and the resulting regression models were used to derive estimates of soil temperature in study sites when instantaneous measurements were not taken. Extrapolated soil temperatures were then applied to regression models generated based on instantaneous R_s and soil temperature for each site using all sampling dates. These models were used to estimate daily R_s for all days between DOY 165 and DOY 294. Across all grassland sites, annual R_s totals based on instantaneous

measurements alone were within 5% of the estimate based on continuous soil temperature data. These results suggested that linear interpolation between the two week sampling intervals provided a good estimate of cumulative R_s for this study.

Statistical Analysis

Statistical analyses were conducted using JMP 6.0 and SAS (SAS Institute Inc.). Changes in cumulative growing season R_s, standing root biomass (C and N), annual root ingrowth (C and N), annual aboveground production C, and the ratio of root ingrowth to aboveground production with time since prairie reconstruction was examined using linear regression with site means (n = 6). The soybean cropping system was excluded from linear regressions for annual root ingrowth and ANPP (n = 5).

Stepwise linear regression was used to identify the strength of soil temperature and soil moisture in explaining seasonal variation in R_s within each age (n = 125 for age 0, n = 150 for ages ≥ 4). Based on the strong significance of soil temperature in explaining seasonal variation in R_s within each age, a two stage analysis was used to test for differences in the effect of soil temperature on R_s among sites. Linear regression was used to model the effect of temperature on R_s within each location for each age (6 ages x 10 locations = 60). General linear model analysis of variance was used to compare model parameter coefficients (e.g. slopes and intercepts) between ages. A similar analysis was conducted using multiple regressions, including both soil temperature and soil moisture within the model. We compared the results of models based on soil temperature with those based on soil temperature and moisture to assess whether differences in the R_s response to soil temperature among ages could be accounted for by soil moisture. A heterogeneous variance ANOVA

model was used for the soybean cropping system to account for unequal variance. Statistical

significance in parameter coefficients was assessed using Bonferroni adjusted p-values. In

all cases, soil respiration was natural log-transformed prior to analyses to meet assumptions

of uniform variance. We used correlation analyses to assess the relationship between

cumulative growing season R_s, root biomass (C and N), annual root ingrowth (C and N), and

annual aboveground production (C) among ages.

RESULTS

Changes in root biomass, root ingrowth, and aboveground production with age

Root biomass showed a significant linear increase with prairie age ($R^2 = 0.89$, P =

0.003), ranging from 17 g C m^{-2} in the soybean crop to 205 C g m^{-2} in the 12 yr old prairie

(Table 2). Root biomass in the soybean crop was similar to the 4 yr old prairie (38 g C m^{-2})

while there was over a threefold increase in biomass from the 4 and 6 yr old prairie (131 g C

m^{-2}). The ratio of carbon to nitrogen in root biomass did not show a linear trend with age,

doubling between the 4 and 6 yr old reconstructions and leveling off among the oldest

reconstructions. Nitrogen content in root biomass showed a similar pattern to that of root

carbon content and increased linearly with age ($R^2 = 0.86$, P = 0.005).

Annual root ingrowth (g C m^{-2}) increased with prairie age, although this trend was not

statistically significant (P = 0.14) (Figure 1). Annual root ingrowth was lowest in the soybean

cropping system (60 g C m^{-2}) and ranged from 89 (age 4) to 202 (age 8) g C m^{-2} among

prairies. A doubling in annual root ingrowth occurred between the 6 and 8 yr old prairies and

then showed a trend toward leveling off, 166 g C m^{-2} (age 9) and 176 g C m^{-2} (age 12).

Within age, seasonal root ingrowth was generally similar in the spring and summer periods

and declined in the fall (Figure 2). However, across ages, there was a pattern toward greater

spring and summer ingrowth in the 8, 9, and 12 yr old sites in comparison to the 4 and 6 yr

old sites and ingrowth in the 8 yr old prairie had a more distinct summer peak.

Root ingrowth tissue chemistry varied considerably both within and among seasons.

The ratio of carbon to nitrogen (C:N) in seasonal root ingrowth tissue ranged from 18 in the

soybean crop (spring) to 83 in the 12 yr old reconstruction (summer) (Figure 2). The C:N

among prairie reconstructions $\geq$ age 4 had a similar range in the spring (30 to 45) and fall (31

to 54) ingrowth periods. The summer C:N ratio ranged from 43 to 83 and showed a strong,

positive increase with age ($R^2 = 0.98$, $P < 0.001$). In contrast to root biomass N content, total

nitrogen in root ingrowth tissue did not show a linear trend with age and ranged from 2.2 to

4.4 g N m^{-2} in the 6 and 8 yr old prairies respectively ($P = 0.28$).

Aboveground production showed no significant linear trend with tallgrass prairie age

($P = 0.22$), ranging from 148 (age 4) to 368 (age 9) g C m^{-2} (Figure 1). The absence of any

discernable trend over time reflected variable spring growth among young and old prairies

(Figure 3). Seasonal production increased sharply between DOY 134 and 178 for the 6, 9,

and 12 yr old prairies, ranging from 217 to 241 g C m^{-2}. This period alone accounted for as

much as 80% of annual production in age 6. Aboveground production in age 4 (95 g C m^{-2})

increased similar to age 8 (98 g C m^{-2}) and accumulated less than half of the biomass

compared to the other prairie ages over the spring time period. From DOY 178 to 226,

production decreased considerably across all prairies compared to the spring period, with

accumulation in the 4, 6, 8, and 12 yr old sites ranging from 46 to 77 g C m^{-2}. Production in

the 9 yr old prairie over the same summer period was 151 g C m^{-2}. In contrast to prairie

reconstructions, the soybean crop accumulated only 33 g C m^{-2} during the spring growth period, with just 10% of the total aboveground crop biomass present by DOY 178. The soybean crop accumulated almost 75% of total biomass, 248 g m^{-2}, within the mid-summer period and productivity increased to 336 g C m^{-2} by DOY 244, a level similar to that of the most productive prairie.

The ratio of root ingrowth to aboveground production did not show any trend with age (P = 0.92). The total of root ingrowth and aboveground production did show an increase with age although it was not significant at the 0.05 level (R^2 = 0.66, P = 0.06). Our estimate of total plant production ranged from 248 to 551 g C m^{-2} yr^{-1} in the 4 and 9 yr old reconstructions, respectively.

Seasonal patterns in soil temperature, soil moisture, and R$_s$

Maximum soil temperatures across all ages occurred between mid July and early August (Figure 4). Mean soil temperatures ranged from 4.1°C in early October within the soybean crop to 26.4°C in early August in the 4 yr old prairie. Mean annual soil temperature ranged from 16.9°C in the 6, 8, and 9 yr old prairies to 17.0°C, 17.4°C, and 17.9°C in prairies age 0, 12, and 4, respectively. Mean soil moisture content (% water volume per soil volume) ranged from 12.1% in the soybean to 39.6% in the 12 yr old prairie (Figure 4). Averaged over all dates, soil moisture content ranged from 27.1% to 30.9% across all sites. Soil moisture was highest in April and generally declined through spring and early summer until reaching seasonal lows between mid-June and mid-July, ranging from 12.1% to 19.4% among all ages. Soil moisture increased from August through the fall, approaching moisture conditions similar to the spring although declining briefly in early October.

The greatest seasonal variability in R_s among prairie ages occurred during the spring and early summer months (Figure 5). At the initiation of R_s sampling (DOY 108), R_s ranged from 1.12 (age 0) to 2.91(age 4) g C m^{-2} day^{-1}. Similar to soil temperature, R_s within each age peaked between mid-July and early-August, with maximum mean daily R_s ranging from 6.20 (age 4) to 8.32 (age 9) g C m^{-2} day^{-1}. R_s in the two oldest prairies (ages 9 and 12) accelerated at a greater rate into the mid-summer months in comparison to the 4, 6, and 8 yr prairies (Figure 5). The soybean cropping system showed a contrasting pattern in seasonal R_s in comparison to the prairie reconstructions. Early spring R_s rates were relatively depressed until mid-June, then rose rapidly and peaked in early August at a time similar to the oldest prairies. R_s rates into the fall, after DOY 243, were less variable among all prairie ages and declined steadily until the end of the sampling period (DOY 320), reaching seasonal lows that ranged from 0.67 in age 8 to 0.96 g C m^{-2} day^{-1} in age 0. Considering these seasonal patterns, cumulative growing season R_s showed a significant, positive increase with grassland age ($R^2 = 0.86$, P < 0.0074), ranging from 624 (age 0) to 939 (age 12) g C m^{-2} gs^{-1} (Figure 6).

Seasonal changes in R_s were strongly related to soil temperature within each prairie age (P < 0.0001) (Table 3). However, seasonal variation in R_s was considerably less predictable with soil temperature in the soybean field ($R^2 = 0.40$). The relationship between R_s and soil temperature was particularly weak in the early spring in the absence of the soybean crop. The addition of soil moisture to soil temperature-based models within each age explained a significant amount of seasonal R_s variation (P < 0.02); however moisture added limited explanatory power. The improvement in model R^2 was greatest in the soybean crop (from $R^2 = 0.40$ to $R^2 = 0.48$) and the 4 yr old prairie (from $R^2 = 0.80$ to $R^2 = 0.90$).

Coefficients of variation (CV) in R_s on a given day averaged 23% with a range from 7% to 66% across sites. The soybean cropping system had the greatest average site level variation (CV 34%), which could be related to the effects of crop management (e.g. soil compaction) on R_s in annual cropping systems (Rochette et al. 1991). These estimates are consistent with reports of a CV of 30% reported for R_s measurements conducted using large chambers (300-500 cm^2) (Davidson et al. 2002).

Predicting R_s across the chronosequence

A comparison of regression slopes from soil temperature-based R_s models showed a significant age effect (P < 0.0001) (Figure 7). There was a trend toward increasing slopes with age, peaking at age 8 and leveling off in the 9 and 12 yr old reconstructions. Pairwise slope comparisons revealed significant differences between the soybean cropping system and prairies ≥ 6 yrs of age (P < 0.0001) and when comparing the 4 yr old prairie to prairies ≥ age 6 (from P < 0.0001 to P = 0.03). Negative slope coefficients indicate that the response of R_s to soil temperature was significantly lower in both the soybean crop and 4 yr old prairie when compared to the prairies ≥ age 6. There was a significant difference in model intercepts among ages (P = 0.017), however we did not compare intercepts for ages with significantly different slopes. Trends in model intercepts among ages with non-significant slopes could be interpreted as a measure of overall seasonal R_s means. Differences in intercept coefficients among prairies ≥ age 6 were no longer significant when pairwise comparisons were Bonferroni adjusted (ranging from P = 0.72 to P = 0.07). Among prairies ≥ age 6, the intercept was distinctively low in the 8 yr old prairie and increased through age 12. Overall,

linear regression models including age, soil temperature, and the interaction term explained

79% of the variation in R_s across the chronosequence.

Comparisons among parameter coefficients from multiple regression models,

including soil temperature and soil moisture, showed that prairie age had a significant effect

on the temperature coefficient ($P < 0.0001$). Temperature coefficients showed a similar

pattern with age in comparison to models based on soil temperature alone. However,

accounting for soil moisture slightly increased the temperature coefficient for each age and

had the greatest effect in the 0 and 4 yr old reconstructions. As a result, after accounting for

soil moisture, soil temperature coefficients were no longer significant when comparing the 4

yr and 6 old prairies ($P = 0.92$). Pairwise comparisons revealed a significant difference in the

R_s response to soil temperature between the 4 yr old prairie and all prairies $\geq$ age 8 ($P <$

0.0001 to $P = 0.017$). Therefore, while holding soil moisture constant, the effect of

temperature on Rs was still significantly lower in the 4 yr old prairie. A comparison of

parameter coefficients from the multiple regression models among the soybean cropping

system and each prairie indicated that the temperature coefficient was no longer significant

(ranging from $P = 0.073$ to $P = 1.00$), in part, a result of a distinct increase in the variance

associated with the soil temperature coefficient in the soybean R_s model.

Comparison of multiple regression R_s models indicated that prairie age had a

significant effect on the soil moisture coefficient ($P = 0.0012$). Pairwise comparisons

indicated a significant difference in the effect of soil moisture on R_s when comparing the 4 yr

to the 6, 9, and 12 yr old prairies ($P = 0.003$ to $P = 0.006$) and differences were less

significant in comparison to age 8 ($P = 0.064$). Parameter coefficients suggest that soil

moisture had a positive influence on R_s in the 4 yr old prairie. Intercept coefficients in

multiple regression models were significantly different (P = 0.017) among ages and showed a similar pattern to models based on temperature alone. However, differences in intercept coefficients when comparing ages 4 and 6, 8 and 9, and 8 and 12 were no longer significant when pairwise comparisons were Bonferroni adjusted (range P = 0.20 to P = 0.27). Overall, the inclusion of soil moisture in regressions based on soil temperature improved model R^2 by 0.04, explaining 83% of the variation in R_s across the chronosequence.

Correlations among cumulative R_s and plant growth

There was a significant, positive correlation between cumulative growing season R_s and root biomass (r = 0.89, P = 0.018) (Figure 8). The positive correlation between R_s and annual root ingrowth was not significant (r = 0.70, P = 0.12), largely because of a relatively low cumulative R_s with high root ingrowth in the 8 yr old prairie (Figure 8). Removal of the 8 yr reconstruction from the analysis resulted in a strong positive correlation between annual root ingrowth and cumulative R_s (r = 0.997, P = 0.0002). ANPP and cumulative R_s were not correlated across prairie ages (r = 0.38, P = 0.46) (Figure 8).

DISCUSSION

It was hypothesized that the establishment and accumulation of belowground plant biomass with early tallgrass prairie development would contribute to increasing rates of R_s with age. Considering that the estimated contribution of root respiration to total R_s in tallgrass prairies may be as high as 40% (Kucera and Kirkham 1971; Craine et al. 1999), greater belowground biomass should lead to an increase in root and rhizosphere respiration

as perennial roots colonize the soil resource and plants allocate carbon belowground to support root growth and maintenance. In addition, root-derived detritus provides the primary source of substrate for microbial decomposition in grasslands and it has been shown that both microbial biomass (Allison et al. 2005) and potential carbon mineralization rates (Camill et al. 2004; Baer et al. 2002) increase with age. However, the changes in the magnitude and controls on R_s across an early tallgrass prairie chronosequence have not been quantified in-situ and are critical to our understanding of the changes in the ecosystem carbon balance with prairie reconstruction. The interaction of age-related changes in above and belowground plant growth and seasonal variation in soil microclimate provided a dynamic set of conditions for predicting R_s across the tallgrass prairie chronosequence.

Changes in plant growth with prairie age

As expected, there was an increase in standing belowground biomass across the chronosequence, consistent with the establishment and accumulation of perennial roots with tallgrass prairie development (Brye et al. 2002). Notably, we observed a threefold increase in root biomass and a twofold increase in the C:N ratio between the 4 and 6 yr old reconstructions. This relatively fast shift toward greater root biomass with lower tissue quality could be related to both the accumulation of dead root biomass and higher N use efficiencies with grassland development (Wedin and Tilman 1990; Craine et al. 2002). For example, a shift in functional dominance within the plant community from C_3 to C_4 grasses has been documented across several young tallgrass prairie plantings (Jastrow 1987; Sluis 2002; Camill et al. 2004). However, contrary to Baer et al. (2002), the increase in root

biomass with age in our study compensated for lower tissue nitrogen concentrations and led to greater accumulation of total N stored in root biomass.

Comparing rates of belowground biomass accumulation with other chronosequence studies in the same geographic region over a similar age gradient are complicated by variability in regional climatic variability, topographic position, and sampling design (e.g. soil depth). However, Baer et al. (2002) reported end-of-the-season root biomass at 1016 g m^{-2} (0-20 cm) in a 12 yr chronosequence of C$_4$ dominated CRP grasslands in Nebraska, a quantity almost double that within our reconstructions (572 g m^2 from 0-30 cm). Allison et al. (2005) reported 582 g m^{-2} (0-5 cm) in an Illinois tallgrass prairie chronosequence after 15 years while suggesting that after 25 yrs root biomass could be approaching equilibrium. Our estimate is also lower than that in native tallgrass prairie in Kansas, where root biomass ranged from 859 to 1086 g m^{-2} (Seastedt and Ramundo 1990). Lower rates of root biomass accumulation compared to these studies could also be attributed to differences in sampling protocol, considering samples in our study were taken in early spring. A relatively low root biomass in this 12 yr chronosequence and a linear trend with age suggests that carbon storage in root biomass has not reached steady state in our grasslands and will likely continue to increase, although not necessarily at a linear rate.

Patterns in root ingrowth and aboveground production with grassland age were more difficult to discern, attributable in part to a distinct increase in root ingrowth and a decrease in aboveground production within the 8 yr old prairie. We can not distinguish this observation from site-specific effects, however, similar to our findings with belowground biomass and the results of Camill et al. (2004), large shifts in root ingrowth occurred over a relatively fast time scale. Our findings indicate these tallgrass prairie reconstructions

produced 1.6 to 3.7 times more belowground plant biomass relative to the soybean cropping system. In addition, a change in tissue chemistry towards increasing C:N ratios during the summer root ingrowth period suggests greater nitrogen use efficiency among older plantings, where more carbon was allocated belowground per unit of nitrogen. This decrease in root tissue quality in older reconstructions has been associated with the increasing dominance of warm season grasses (Baer et al 2002). Interestingly, a consistently low C:N ratio in soybean roots led to only a 20% increase in root ingrowth nitrogen after 12 yrs, despite a threefold increase in carbon inputs. High carbon to nitrogen ratios in roots has been correlated with lower rates of root respiration per unit of production, greater root longevity, and a decrease in availability for soil microbial decomposition (Craine et al. 2002), while also correlated with positive rates of soil organic matter accumulation (Knops and Tilman 2000; Russell et al. 2004).

Our annual root ingrowth estimate in the oldest prairies ($\geq$ age 8, 458-577 g m^{-2} yr^{-1}) is on the high end of estimates of root production through various methods summarized by Johnson and Matchett (2001) for studies in native tallgrass prairie (202 to 429 g m^{-2} yr^{-1}). Our observation of a large shift in root ingrowth among prairies $\leq$ age 6 and $\geq$ age 8 combined with estimates of root ingrowth in mature prairies (Johnson and Matchett 2001) suggests that root growth in our reconstructions within 12 years is comparable to that of native tallgrass prairie. Our annual estimate for soybean (156 m^{-2} yr^{-1}) is considerably lower than that reported for soybean in Missouri (478 g m^{-2} yr^{-1}) (Buyanovsky and Wagner 1986). Lower than average annual precipitation, particularly in the early spring and summer, and variability in soybean cultivars could potentially explain some of this variation.

The range of aboveground production across all sites (345-856 g m^{-2}) is generally higher than the 20 yr average of ungrazed tallgrass prairie (416 g m^{-2}) reported by Knapp et al. (1998) and the regional average of 560 g m^2 summarized by Risser et al. (1981). However, our estimates are comparable to those reported in young grasslands recently planted into cultivated land (Dornbush and Raich 2006; Baer et al. 2002; Baer et al. 2003).

While the ratio of aboveground to root ingrowth showed no trend with age, our results suggest that our reconstructions were highly productive both above and belowground within 12 years. Most notably, annual aboveground production among the oldest reconstructions (ages 9 and 12) was similar to that of the soybean cropping system despite a roughly threefold increase in root ingrowth rates. The percentage of root ingrowth relative to total production in our prairie reconstructions (31% to 59%) is within the range reported for temperate grasslands (Gower et al. 1999) while that within the soybean field was approximately 17%, compared to 45% reported by Buyanovsky and Wagner (1986).

Total plant production increased across the chronosequence with a maximum of 1304 g m^{-2} yr^{-1} in the 9 yr old prairie. This estimate is within the range of NPP reported by Risser (1991), 850 to 1350 g m^{-2} yr^{-1}, for grasslands within our geographic region. In the case of soybean, Buyanovsky and Wagner (1986) reported a net annual production of 1309 g m^{-2} yr^{-1} compared to a value of 938 g m^{-2} yr^{-1} in our study. Although our relatively shallow sampling depth (30 cm) could underrepresented root growth, particularly in older prairies, reports in native tallgrass prairie suggest that 70-90% of total root biomass is found within the top 30 cm of the soil profile (Dahlman and Kucera 1965; Rice et al. 1998; Kucharik et al. 2006).

Relationship between R$_s$, temperature, and moisture across the chronosequence

Seasonal variation in R$_s$ was strongly related to seasonal changes in soil temperature across prairie reconstructions, similar to studies in native tallgrass prairies (Mielnick and Dugas 2000; Knapp et al. 1998; Kucera and Kirkham 1971, Dugas et al. 1999), older prairie reconstructions (Wagai et al. 1998; Kucharik et al. 2006) and young grassland plantings (Dornbush and Raich 2006). While low soil moisture has the potential to limit R$_s$ and confound predictions based on soil temperature, particularly at high temperatures, soil moisture explained little additional variation within these reconstructed prairies. Despite a distinctly dry spring and early summer, there was no evidence that soil moisture presented strong limitations to R$_s$. However, we did find evidence for differences in the response of R$_s$ to soil moisture among prairies. The significant positive R$_s$ response to soil moisture in the youngest prairie (age 4) could have been related to the effects of aboveground plant biomass on soil microclimate. Limited aboveground cover (considering both live vegetation and dead litter) in younger reconstructions could lead to decreased interception rates and a lower buffering capacity to both soil wetting and subsequent surface evaporation (Knapp and Seastedt 1996) Therefore, R$_s$ in the 4 yr old prairie would have been more responsive to seasonal precipitation events and more extreme fluctuations in soil moisture. Specifically, it appears that a midsummer increase in soil moisture corresponded with an increase in R$_s$ in the youngest prairie. These results are consistent with Knapp et al. (1998), where the effects of irrigation were observed from July through September, although increasing maximum monthly R$_s$ by only 8%.

In comparisons of seasonal R$_s$ patterns among ages, the slope of soil temperature-based R$_s$ models varied across the chronosequence, indicating the importance of age-related

site characteristics to predict seasonal R_s differences across the chronosequence. The trend toward a greater response of R_s to soil temperature with age was significant in comparisons among the youngest (4 yr old) and oldest prairies (ages 8, 9, 12). In general, the slope of the R_s by soil temperature regression increased along the chronosequence until the 8 yr old reconstruction and leveled off in ages 9 and 12. In another comparative study among tallgrass prairies in Wisconsin, Kucharik et al. (2006) cited the importance of different soil moisture conditions in accounting for seasonal differences in the R_s response to soil temperature, however our results suggested that after accounting for surface soil moisture conditions across sites, the trend toward increasing slopes with age remained and there were still significant differences among the youngest and oldest reconstructions.

Relative to the prairie reconstructions, seasonal soil temperature and moisture were relatively poor predictors of R_s within the soybean cropping system, suggesting that abiotic parameters alone are insufficient for predicting intra-annual variation in R_s at that site. Others have reported similar findings in annual cropping systems (Wagai et al. 1998; Han et al. 2007), where changes in seasonal R_s were not reflected in soil temperature and R_s predictions have been improved with the inclusion of a measure of substrate supply (Han et al. 2007). Han et al. (2007) modeled intra-annual R_s in a corn crop based on the interaction of soil temperature and plant activity and found that the seasonal temperature response of R_s could be predicted using soil temperature and both plant biomass and NPP, which were correlated with intercept and slope parameters respectively. Similarly, our results suggest that low root biomass and activity in the soybean site in the early spring potentially limited the rhizosphere contribution to total soil respiration at the beginning of the season (Rochette and Flanagan

1997) and could have detached the otherwise strong relationship between soil temperature and soil respiration across the chronosequence.

Changes in cumulative R_s across the chronosequence

To our knowledge, no previous study has assessed the relationship between age and soil respiration in early tallgrass prairie reconstructions. Brye et al. (2002) found no differences in annual soil respiration in tallgrass prairie reconstructions from age 19 to 24 while Dornbush and Raich (2006) reported greater annual R_s in older grasslands among C_3 and C_4 dominated plantings. Our results indicate that growing season R_s increased by 64% over the 12 yr chronosequence at a rate of approximately 27 g C m^{-2} yr^{-1}. This study suggests that changes in growing season R_s with prairie age were strongly related to the interaction of soil temperature, root biomass accumulation, and seasonal root activity.

These findings are consistent with a number of studies that have found strong relationships between root biomass and R_s across grasslands at local scales (Craine and Wedin 2002; Kucera and Kirkham 1971; Dornbush and Raich 2006; Dugas et al. 1999). The increasing response of R_s to soil temperature across the chronosequence was likely a factor of both the direct effects of temperature on root and rhizosphere metabolism and an increase in root respiration associated with greater spring and summer root growth in older prairies. This finding is consistent with those of Boone et al.(1998), where the temperature sensitivity of roots and the associated rhizosphere was found to be greater than the bulk soil; however it is difficult to distinguish between the contribution of greater specific root respiration (CO_2 produced per g of tissue) and the direct effects of temperature (Davidson et al. 2006). Similar to Dornbush and Raich (2006) and in contrast to studies that have suggested a relationship

between canopy characteristics and R_s (Norman et al. 1992; Bremer and Ham 2002; Craine et al. 1999; Franzluebbers et al. 2002) we found no correlation between cumulative R_s and aboveground production across the chronosequence. This result could be attributed to the lack of any consistent trend between aboveground and belowground plant growth across the chronosequence.

We also expected a strong relationship between cumulative R_s and annual root ingrowth across the chronosequence, in part, associated with the contribution of root and rhizosphere respiration to total soil respiration in developing prairies. The 8 yr old reconstruction detached an otherwise strong, positive relationship between annual root ingrowth and cumulative R_s across the chronosequence, where consistently high seasonal ingrowth rates were not reflected in seasonal R_s. Given the lack of true replication this observation is difficult to explain and could be an artifact of site-specific conditions or management history. These findings also support recent analyses that suggest using the temperature response of R_s (i.e. Q_{10}) to predict the seasonality of R_s can be misleading (Davidson et al. 2006). The slope of soil temperature-based R_s model in the 8 yr old reconstruction appeared to be an artifact of lower basal respiration, with low R_s rates in early spring rather than greater R_s rates at high temperatures.

Differences in root tissue chemistry, as found in our study, have shown to influence rates of decomposition in both soybean cropping systems (Cheng et al. 2003; Broder and Wagner 1988) and perennial grasslands (Wedin and Tilman 1990). High nitrogen content in root tissues has shown to have a positive rhizosphere priming effect in soybean and contributed to high seasonality in total R_s (Cheng et al. 2003) with greater rates of soil organic matter decomposition relative to other annual crops (Cheng et al. 2003; Broder and

Wagner 1988). High decomposition rates in combination with low inputs in soybean have suggested limited carbon accumulation rates in these systems (Huggins et al. 2007). In our study, cumulative Rs rates per unit of annual root ingrowth ranged from 10 to 3.7 in the soybean crop and 8 yr old prairie, respectively.

Comparison of our cumulative estimate with other studies is limited because we did not account for winter R_s. Assuming winter R_s is not zero, linear interpolation between end-of-season R_s to early spring R_s rates produces a maximum annual R_s estimate of 1197 g C m^{-2} yr^{-1} in the 12 yr old prairie. This estimate is on the low end of the 1229 and 1428 g C m^{-2} yr^{-1} range reported by Kucharik et al. (2006) in a Wisconsin tallgrass prairie remnant and a 60 yr old reconstruction respectively, as well as the range of 1.3 to 2.1 kg C m^{-2} yr^{-1} reported from native tallgrass in Kansas (Knapp et al. 1998). However, this estimate is similar to the maximum annual R_s (1260 g C m^{-2} yr^{-1}) reported from slightly younger C_4 dominated Iowa grasslands (Dornbush and Raich 2006).

Conclusion

Changes in root biomass and root ingrowth with tallgrass prairie age revealed that these young reconstructions were highly productive belowground within 12 years, especially in comparison to the soybean cropping system. Cumulative R_s increased with tallgrass prairie age and R_s represented the greatest carbon flux across the chronosequence. We found a strong positive correlation between cumulative R_s and root activity with the development of these young grasslands in formerly cultivated land. These changes in belowground plant development with age influenced soil temperature-based R_s predictions, while there was no relationship between R_s and aboveground production. However, among age variation in

belowground plant biomass was far greater than that of cumulative R_s and a threefold increase in root ingrowth and an eightfold increase in standing root biomass were accompanied by only a 50% increase in cumulative R_s. Therefore, our study suggests an overriding influence of soil temperature in predictions of intra-annual R_s across these young tallgrass prairie reconstructions while improving R_s predictions will require the direct assessment of belowground plant activity. Clearly, further study is required to consider these trends over longer time scales and with intra-annual climatic variability. Future study should also focus on the separation of root and microbial respiration within reconstructions in an effort to differentiate the response of these component fluxes with prairie development.

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APPENDIX

Diurnal soil respiration rates and soil temperature (10 cm) on (a) 22 June 2006 (DOY 173)
and (b) 2 November 2006 (DOY 306) at Neal Smith National Wildlife Refuge, Iowa. Values
represent the mean (± 1 standard error) of four locations measured within each hour and the
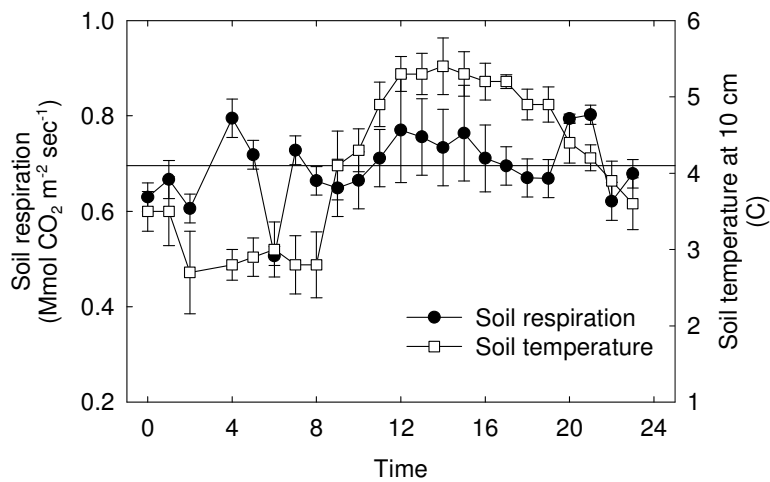horizontal line represents the daily average soil respiration for each day.

(a)
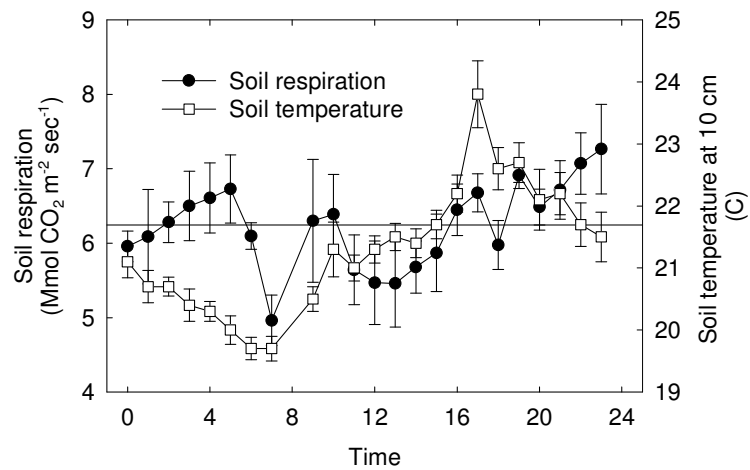
(b)

FIGURE LEGENDS

Figure 1: Changes in annual root ingrowth (0-30 cm) and aboveground production among a soybean cropping system (age 0) and five different-aged prairie reconstructions in the 2006 growing season at Neal Smith National Wildlife Refuge, Iowa. The soybean cropping system (age 0) is represented for comparison only and values represent the mean of 10 locations within each age.

Figure 2: Seasonal patterns in root ingrowth C (0-30 cm) and the C:N ratio of root ingrowth tissues among a soybean cropping system (age 0) and five different-aged prairie reconstructions in the 2006 growing season at Neal Smith National Wildlife Refuge, Iowa. Values represent the mean of 10 locations within each age.

Figure 3: Patterns in cumulative aboveground production for a soybean cropping system (age 0) and five different-aged prairie reconstructions in the 2006 growing season, Neal Smith National Wildlife Refuge, Iowa. Values shown represent the mean of 8 locations within each age and the soybean cropping system (age 0) is represented for comparison only.

Figure 4: Seasonal patterns in soil temperature (10 cm) and volumetric soil moisture content (% water volume per soil volume) from 0-6 cm in a soybean cropping system (age 0) and five different-aged prairie reconstructions during the 2006 growing season at Neal Smith National Wildlife Refuge, Iowa. Values shown represent the mean of 10 locations within each age.

Figure 5: Seasonal pattern in mean daily and mean cumulative soil respiration for a soybean cropping system (age 0) and five different-aged prairie reconstructions during the 2006 growing season at Neal Smith National Wildlife Refuge, Iowa. Values shown represent the mean of 10 locations within each age.

Figure 6: The relationship between age of reconstructed prairie (age 0 = soybean) and cumulative growing season (April through November) soil respiration in 2006 at Neal Smith National Wildlife Refuge, Iowa. Values shown represent the mean of 10 locations within each age.

Figure 7: The relationship between soil temperature (10 cm) and soil respiration (natural log transformed) based on seasonal instantaneous measurements in a soybean cropping system (age 0) and five different-aged prairie reconstructions from May through November 2006 at Neal Smith National Wildlife Refuge, Iowa. Lines represent best fit linear regressions and values shown represent the mean of 10 locations for each measurement date within each age.

Figure 8: Correlations between cumulative growing season (gs) soil respiration (May through November) and root biomass C (0-30 cm), annual root ingrowth C (0-30 cm), and annual aboveground production C for a soybean cropping system (age 0) and five-different aged prairie reconstructions at Neal Smith National Wildlife Refuge, Iowa 2006. Values shown represent site means for each age.
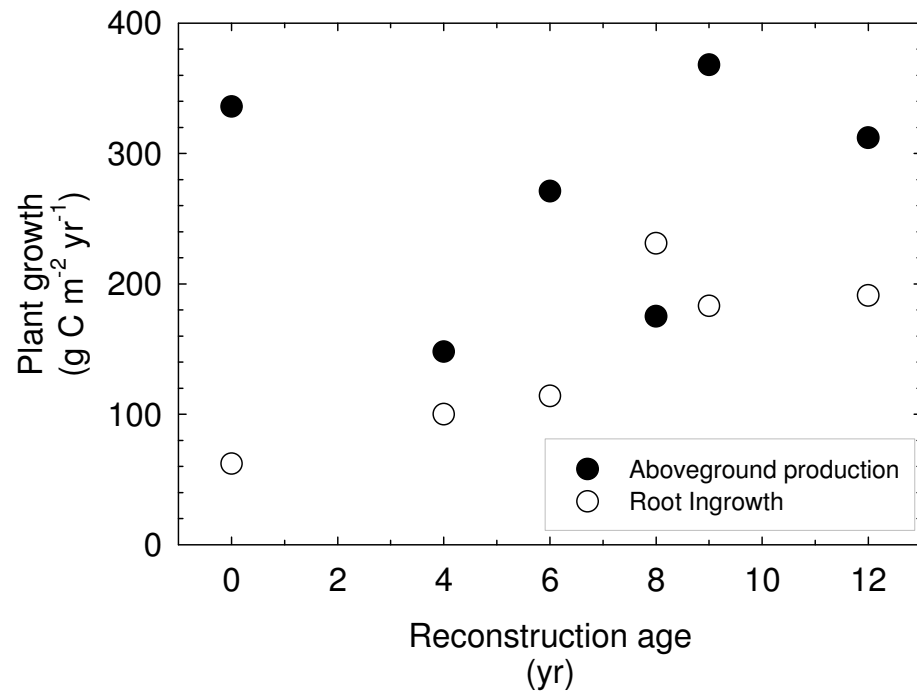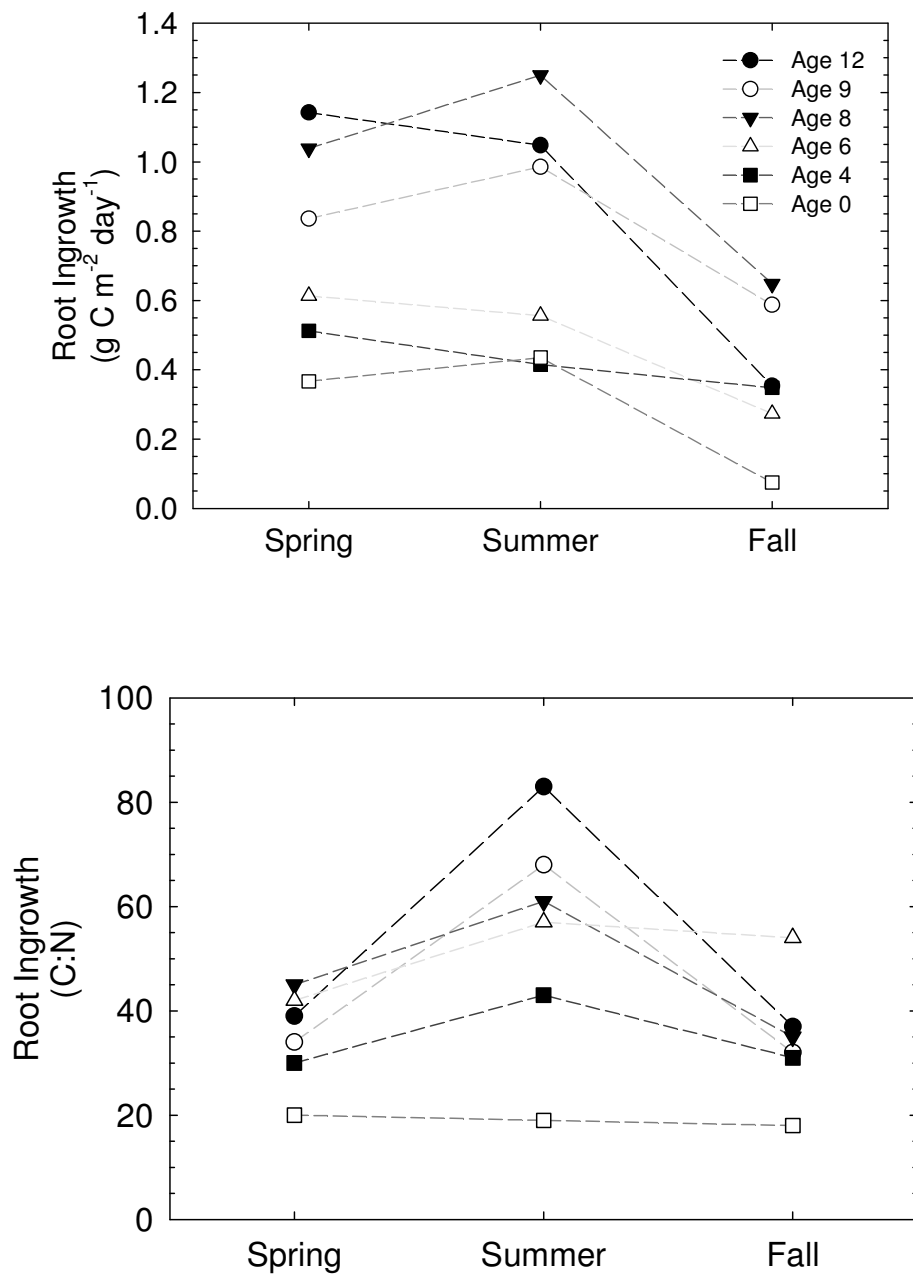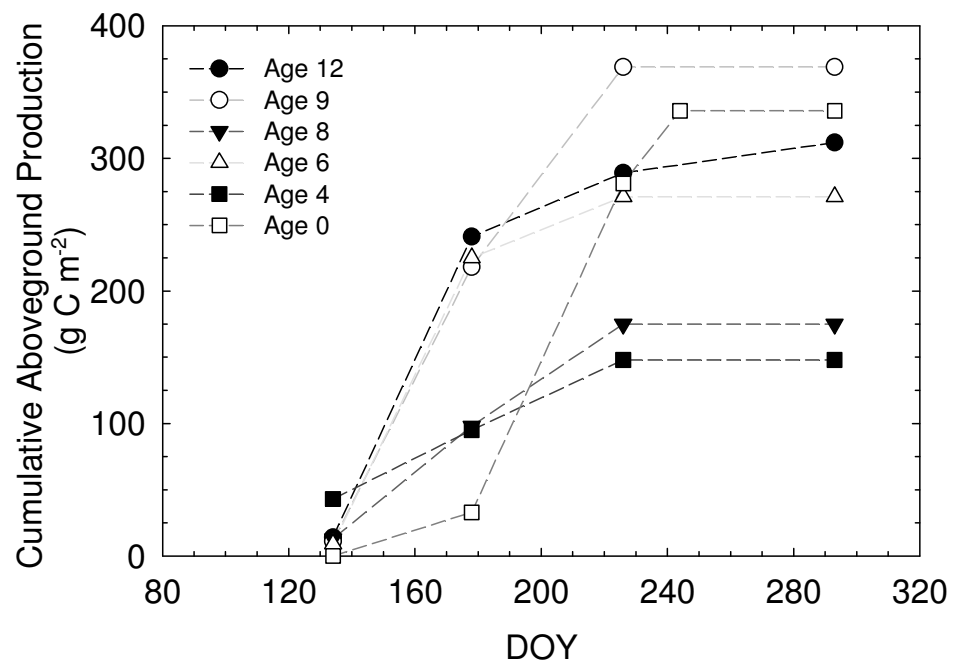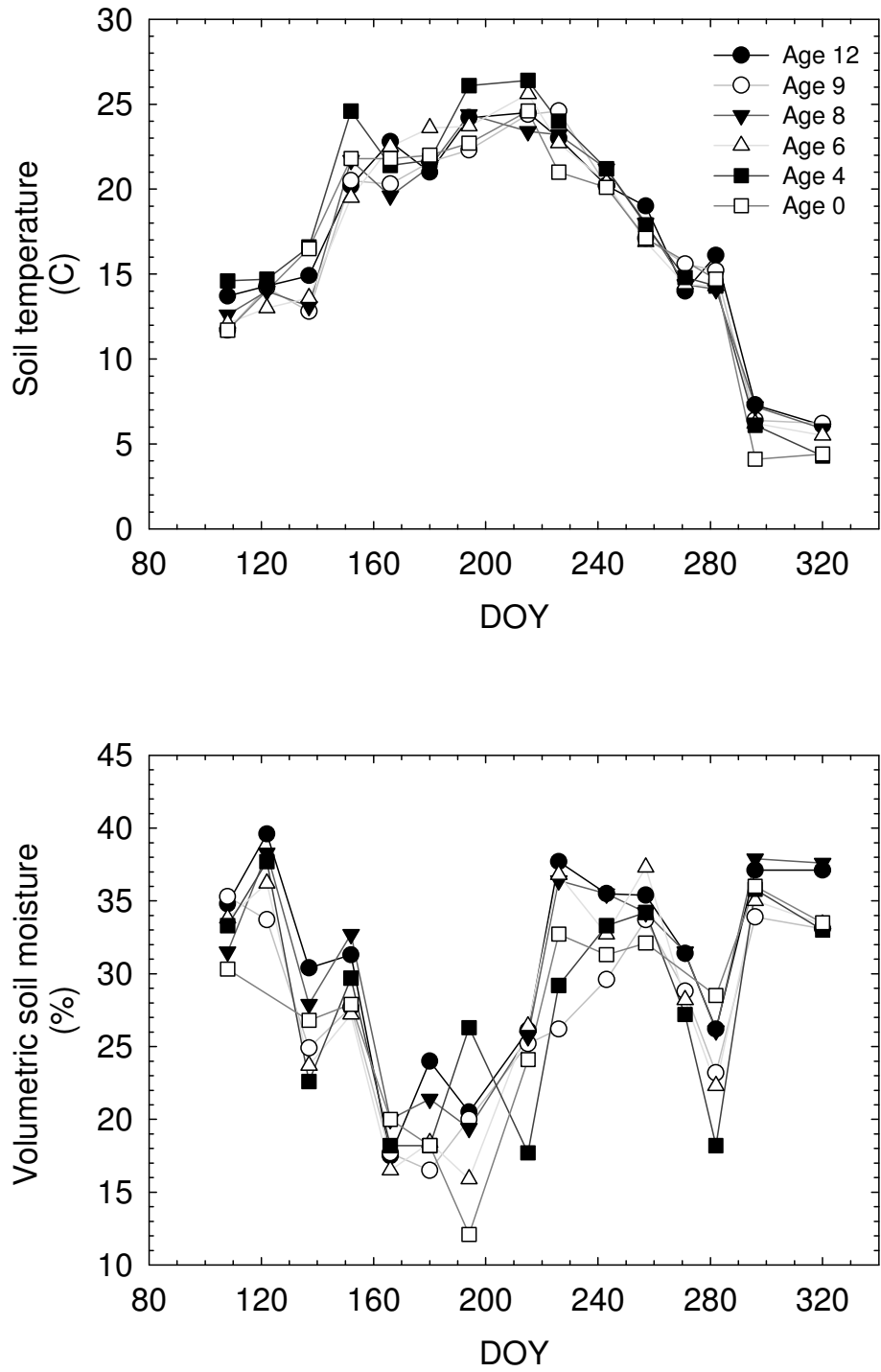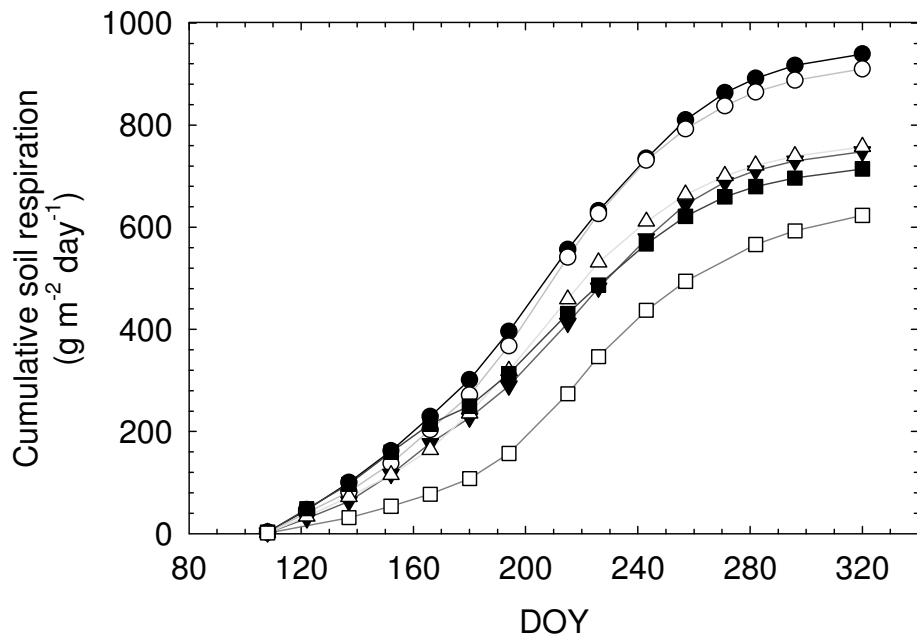
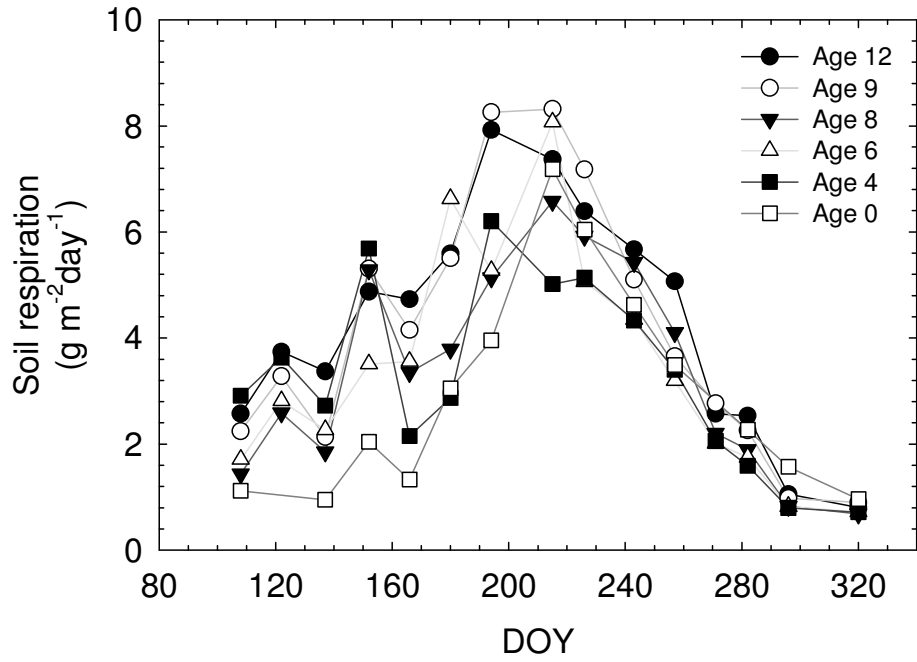FIGURES

Figure 1:

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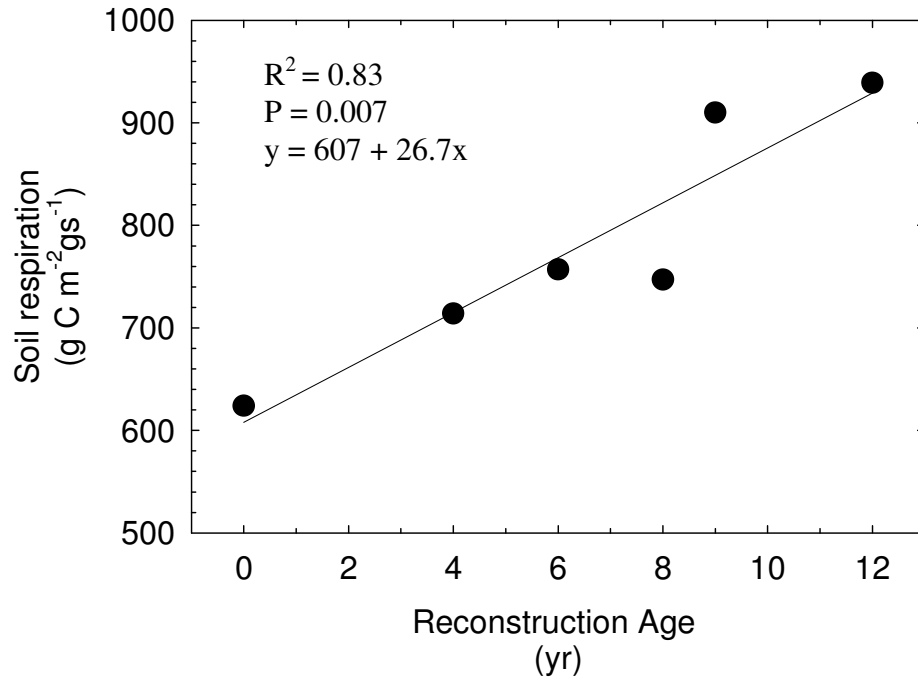
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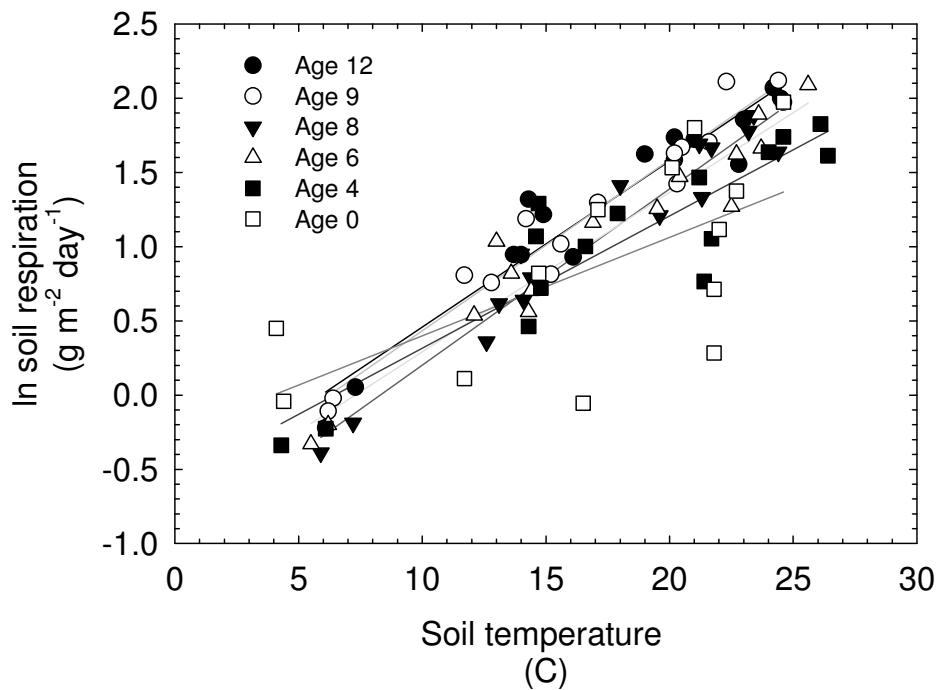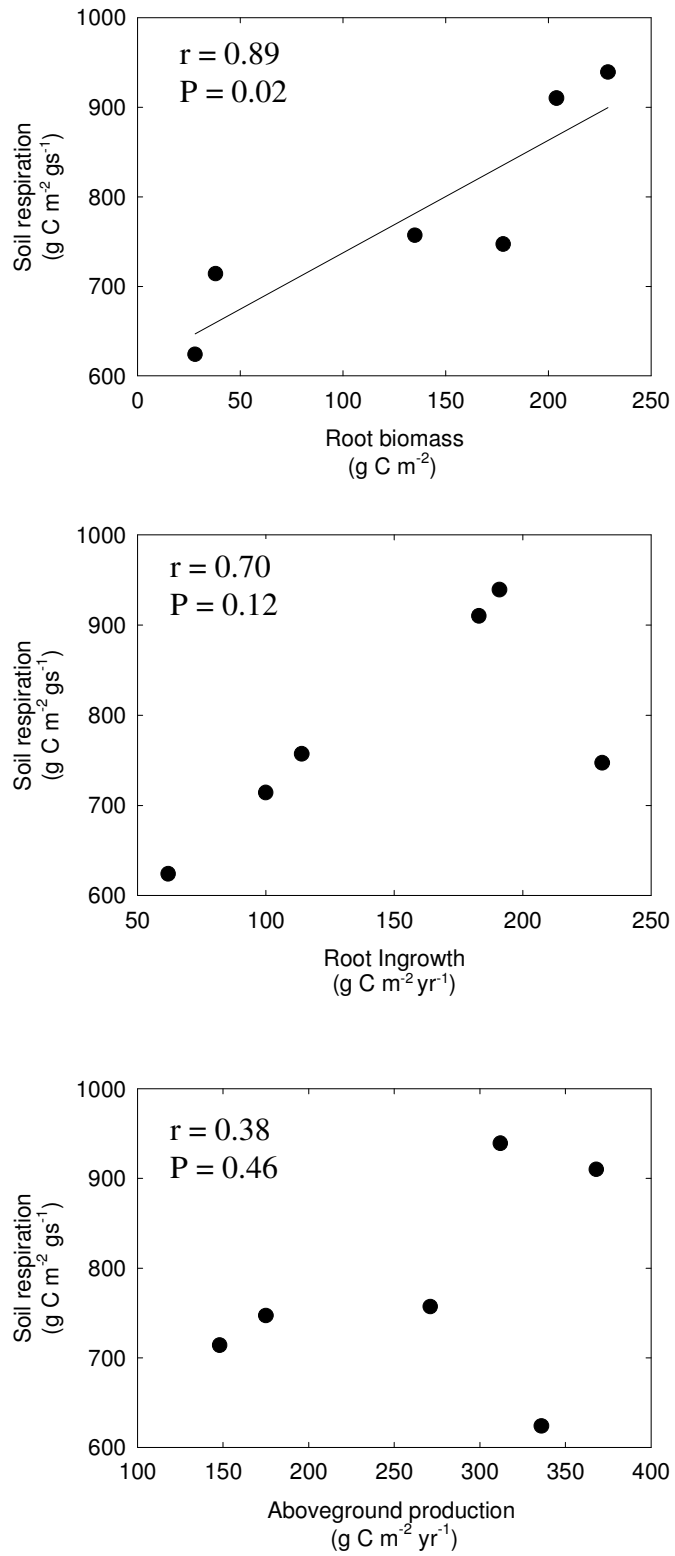
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TABLE LEGENDS

Table 1: Soil carbon and nitrogen content and soil physical characteristics for a soybean cropping system (age 0) and five different-aged prairie reconstructions at Neal Smith National Wildlife Refuge, Iowa. Values represent the mean of locations for each site and correspond to a 0-30 cm sampling depth (Cambardella, unpublished). *Represents missing data.

Table 2: Changes in spring root biomass (0-30 cm) parameters with age of prairie reconstruction (age 0 = soybean) in 2006 at Neal Smith National Wildlife Refuge, Iowa, including simple linear regression (SLR) equations, coefficient of determination, and significance level. Biomass values represent the mean of 10 locations within each age.

Table 3: Linear regression models describing the relationship between soil temperature (10 cm) and soil respiration for a soybean cropping system (age 0) and five-different aged prairie reconstructions, Neal Smith National Wildlife Refuge, Iowa. Regressions are based on biweekly instantaneous measurements over the 2006 growing season (n = 125 for age 0 and n = 150 for age 4-12).

TABLES

Table 1:

| Reconstruction Age (yr) | % C | % N | C:N | Soil Texture | | |
				% Sand	% Silt	% Clay
0	3.0	0.26	11.6	2.4	67.6	30.0
4	2.7	0.26	10.3	1.9	65.8	32.3
6	2.5	0.24	10.3	2.6	67.6	29.2
8	3.6	0.32	11.1	2.7	68.3	29.0
9	3.2	0.29	10.8	2.7	66.9	30.5
12	3.1	0.28	10.8	2.4	67.6	30.0

Table 2:

Reconstruction Age (yr)	Root Biomass (g m^{-2})	Root C:N	Root Biomass C (g m^{-2})	Root Biomass N (g m^{-2})
0	48	24	17	0.7
4	96	49	38	0.8
6	337	97	131	1.6
8	445	83	164	2.0
9	511	71	186	2.7
12	572	77	205	2.8
SLR	10.2 + 49.9x		6.8 + 17.9x	0.45 + 0.2x
R^2	0.89		0.89	0.86
P value	0.003	0.1	0.003	0.005

Table 3:

Reconstruction Age (yr)	Model	R^2	P value
0	$\ln (R_s) = -0.4855 + 0.0751(T)$	0.40	<0.0001
4	$\ln (R_s) = -0.6033 + 0.0899(T)$	0.80	<0.0001
6	$\ln (R_s) = -0.8017 + 0.1068(T)$	0.85	<0.0001
8	$\ln (R_s) = -1.0469 + 0.1209(T)$	0.91	<0.0001
9	$\ln (R_s) = -0.7453 + 0.1152(T)$	0.88	<0.0001
12	$\ln (R_s) = -0.6863 + 0.1124(T)$	0.88	<0.0001

CHAPTER 3: GENERAL CONCLUSIONS

CONCLUSIONS

Our study of plant growth and soil respiration across a 12 yr chronosequence of tallgrass prairie reconstructions quantified how reconstructions alter the magnitude and rate of soil carbon cycling processes when planted into formerly cultivated land. As expected, the planting of young grasslands resulted in relatively fast changes in the accumulation of belowground biomass and the quantity of carbon and nitrogen stored within roots increased across the chronosequence. While patterns in belowground plant growth were more difficult to predict, reconstructions were highly productive and root ingrowth in older reconstructions was comparable to ingrowth estimates from native tallgrass prairie (Johnson and Matchett 2001). Our results also provided a measure of plant carbon allocation in prairie reconstructions relative to a soybean cropping system. For example, estimates of belowground plant growth in the oldest (12 yr) reconstruction were three times that of the soybean crop, despite relatively similar rates of aboveground production. Contrary to our hypothesis that the ratio of belowground plant growth to aboveground production would increase with age, changes in plant allocation were more dynamic and much less predictable, although total plant production increased with age.

Our results showed seasonal differences in the tissue chemistry of root inputs, where the soybean crop had consistently high quality (low C:N ratio) inputs and an decrease in the quality of root inputs among older reconstructions was most pronounced during the summer growth season. Results suggest differences in nitrogen use efficiency among reconstructions,

where older plantings allocated more carbon belowground per unit of nitrogen. Other studies have documented a similar trend with an increase in warm season grass dominance in young reconstructions (Camill et al. 2004). Lower quality root tissues have been associated with lower rates of root respiration per unit of production, greater root longevity, and a decrease in availability for soil microbial decomposition (Craine et al. 2002), traits that could influence rates of soil carbon accumulation in grassland reconstructions (Knops and Tilman 2000).

These changes in belowground plant development with tallgrass prairie age influenced cumulative soil respiration and soil temperature-based soil respiration predictions. Cumulative soil respiration represented the largest flux of carbon across the chronosequence and increased with tallgrass prairie age. We found no correlation between aboveground production and cumulative soil respiration while this increase was strongly related to the accumulation of belowground biomass with age. An increase in root biomass with age could lead an increase in both the root and rhizosphere and the microbial contribution to total soil respiration.

While soil temperature explained a majority of the seasonal variation in soil respiration within each reconstruction, our results suggest that tallgrass prairie age and increasing root activity within older reconstructions also had an effect on the seasonal variation in soil respiration. The interaction between soil temperature and soil respiration among ages was most dramatic in the soybean cropping system with the absence of perennial root biomass. This study suggests an overriding influence of soil temperature in predicting intra-annual soil respiration across these young reconstructions while improving soil respiration predictions will require the direct assessment of belowground plant activity.

RECOMMENDATIONS FOR FUTURE RESEARCH

Growing concerns over the future increase in atmospheric CO_2 concentrations and attention towards the design of multifunctional agricultural landscapes will likely increase incentives towards the reconstruction of grassland ecosystems. Therefore, there is a demand to both quantify soil carbon stocks and fluxes in reconstructed grasslands and to predict the future potential for grasslands to accumulate soil carbon. Given the conclusions of this research the following research directions are advised:

1) Our understanding of tallgrass prairie development will require long-term monitoring beyond the time frame considered within this study to document changes in plant allocation, particularly belowground, and soil respiration while accounting for annual climatic variability. Incorporating the impact of disturbance, including fire and grazing, in long-term studies could also provide insight into interaction of age and management practices in determining changes in belowground carbon allocation with tallgrass prairie development;

2) Soil respiration represents the total of root and rhizosphere respiration and the turnover of soil carbon through microbial respiration. Therefore, soil carbon budgets in reconstructed grasslands based on measurements of soil respiration will require research towards distinguishing the relative contribution of these different sources and how this contribution changes over time. Similarly, work towards the separation of heterotrophic and autotrophic sources could elucidate differences in the importance of climatic and biological controls on

these two processes that otherwise complicate the interpretation of soil respiration and predictions to environmental change;

3) A mechanistic understanding of soil carbon accumulation in grassland reconstructions requires research towards the potential for negative feedbacks associated with plant growth and development and microbial activity. For example, attention could be given to the interaction between low quality root inputs, soil nitrogen dynamics, and the effects on the contribution of microbial activity to total soil respiration in reconstructed grasslands.

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